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Molecular Mechanisms of Heart Muscle Disease

SUBCELLULAR MECHANISMS of dilated heart muscle disease (congestive cardiomyopathy) are poorly understood. With the development of techniques of molecular biology, some causal events in a specific form of congestive cardiomyopathy, the form induced by the anthracycline doxorubicin (Adriamycin) hydrochloride, have recently been elucidated. Postulated mechanisms of doxorubicin congestive cardiomyopathy include DNA intercalation, free-radical injury, lipid peroxidation, and mitochondrial damage. It has been shown that a relatively specific effect of doxorubicin use is selective changes in the expression of cardiac α-actin (α_c-actin) polypeptide and messenger RNA in vitro and by analogous studies in vivo. This selective defect in the expression of a critical sarcomeric protein may ultimately relate to the defective contractility in doxorubicin congestive cardiomyopathy.

Data show that doxorubicin has a relatively selective effect on the steady-state expression of α_c-actin mRNA compared with nonsarcomeric β-actin or glyceraldehyde-3-phosphate dehydrogenase mRNAs in the rat heart in vivo in both a dose-related and a temporal way in which the nadir acute doxorubicin effect occurred three days after therapy. It has been known for years that doxorubicin congestive cardiomyopathy is related to cumulative doxorubicin therapy. Many antineoplastic chemotherapy regimens use sequential doxorubicin administration, which may empirically correlate with recent in vivo- and in vitro-derived scientific data.

The defect in the expression of α_c-actin mRNA induced experimentally by doxorubicin may relate to cumulative toxic effects. We have initiated an experimental model for recovery of the heart from doxorubicin's cardiotoxic effects. In this model, cardioprotective agents are used to prevent doxorubicin cardiotoxicity. Preliminary data suggest that the effect of the drug on α_c-actin mRNA expression cannot be ablated by administering some cardioprotectant agents such as ICRF 187.

Molecular biologic methods have been applied more recently to the emerging problem of congestive cardiomyopathy in the acquired immunodeficiency syndrome (AIDS) to explore its subcellular mechanisms. Preliminary data suggest that zidovudine (AZT), the widely used antiretroviral drug, has an associated cardiotoxicity in rats that is manifested ultrastructurally by mitochondrial disarray in cardiac myocytes. The doses used in the experimental system were relatively high compared with those used in AIDS therapy at present. Future work will explore the molecular mechanisms of AZT cardiotoxicity by attempting to localize the molecular targets that may correlate with the observed cardiac ultrastructural changes induced by AZT by using methods adapted from doxorubicin-induced congestive cardiomyopathy models.

The pathogenetic mechanism of drug-induced congestive cardiomyopathy may not directly relate to a depressed expression of α_c-actin mRNA in the heart. Nonetheless, it is intriguing to find that depressed α_c-actin mRNA expression in the myocardium appears to relate pharmacologically to doxorubicin administration. Future studies may link doxorubicin-induced congestive cardiomyopathy mechanistically with defects in the expression of sarcomeric proteins and mRNAs, particularly α_c-actin. Studies of models of the disorder provide a better understanding of the molecular mechanisms that are involved in the regulation of myocardial actin homeostasis in health and potential derangements in heart muscle disease.

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Hepatitis C Test

NON-A, NON-B HEPATITIS, defined as hepatitis resulting from infection with agents other than the hepatitis A or B viruses, is the most frequent transfusion-associated infection in the United States and a major cause of transfusion-related morbidity and mortality. Studies in the late 1970s showed that non-A, non-B hepatitis developed in as many as 10% of transfusion recipients. Although the acute infection is usually asymptomatic, chronic liver disease will develop in 50% of infected patients, and in 20% of these, cirrhosis may develop. In the absence of a specific test for the causative agent of non-A, non-B hepatitis, blood banks in the mid-1980s began screening donors for elevated levels of alanine aminotransferase (formerly glutamic-pyruvic transaminase) and antibody to the hepatitis B core protein. These tests were predicted to identify 30% to 40% of infectious blood donors.

Transmissivity studies in animals indicated that the major causative agent of non-A, non-B hepatitis was a small RNA virus, although many attempts to isolate this agent were unsuccessful. Recently investigators at the Chiron Corporation and the Centers for Disease Control took a novel approach and synthesized components of this virus using recombinant DNA technology. The investigators isolated nucleic acid from the plasma of a chimpanzee with non-A, non-B hepatitis and inserted pieces of this material into bacteria. Of 1 million recombinant bacterial colonies screened, 1 expressed a protein recognized by antibodies from patients with non-A, non-B hepatitis. The genetic insert in this colony was identified, and a larger genetic clone containing this sequence was then inserted into yeast. The recombinant protein produced by this yeast, called the C100-3 protein, is the basis for the hepatitis C virus (HCV) screening test licensed by the Food and Drug Administration (FDA) on May 2, 1990.

The HCV screening test detects antibodies that bind to the C100-3 protein. A large majority of patients with chronic non-A, non-B hepatitis, both transfusion-associated and non-transfusion-associated, have antibodies to this protein. The

test has limited value, however, in the diagnosis of acute hepatitis. Antibodies to the C100-3 protein do not appear until an average of 22 weeks from the time of infection and may not appear for a year or longer. Some patients may lose antibody to this protein at different phases of their liver disease.

Blood banks instituted the HCV screening test as soon as it was licensed by the FDA. Approximately 0.4% to 1.5% of volunteer blood donors have a reaction with this test. It is estimated that the use of this screening test will further decrease the rate of transfusion-transmitted non-A, non-B hepatitis by at least 50%.

False-positive reactions on the HCV screening test occur. Research confirmation tests, available from the manufacturers of the screening tests, indicate that as many as 40% to 60% of the reactions in blood donors are false-positive—that is, unrelated to hepatitis C. Among populations at high risk for non-A, non-B hepatitis, however, such as persons with hemophilia and intravenous drug users, almost all reactions are confirmed. Preliminary data suggest that the results of these confirmation tests correlate with infectivity.

Preliminary epidemiologic studies using the HCV screening test indicate that there is a low risk of nonparenteral viral transmission but that there may be an association of hepatitis C with cirrhosis, hepatocellular carcinoma, and possibly "idiopathic" liver diseases. These issues will have to be examined more closely in conjunction with the use of confirmatory tests.

Investigators have now successfully sequenced the entire genome of hepatitis C, which they have established to be a single-stranded RNA virus with a structure similar to flaviviruses. Other regions of the viral genome are now being investigated in search of other immunogenic viral components.

It is remarkable that this infectious agent that has so long eluded isolation or even visualization has finally been characterized. Although there are other agents which can cause non-A, non-B hepatitis, this recent accomplishment will go a long way toward improving the safety of the blood supply and our understanding of the epidemiology and natural history of the major form of non-A, non-B hepatitis.

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Epstein-Barr Virus in Hodgkin's Disease

ON THE BASIS OF epidemiologic and serologic studies, Epstein-Barr virus has long been suspected to be implicated in the pathogenesis of Hodgkin's disease. Recently researchers used molecular hybridization techniques and in situ hybridization to demonstrate the presence of Epstein-Barr viral DNA in about 20% of tissues involved by Hodgkin's disease. Other investigators have used Southern blot testing to show Epstein-Barr viral DNA in between 16% and 29% of patients with Hodgkin's disease. Analyses of cases of Hodgkin's disease by dot blotting and polymerase chain reactions have generally produced similar results, except for a preliminary study in which about 60% of patients with lymphocyte-predominant Hodgkin's disease were found to have cells whose DNA contained Epstein-Barr virus-related sequences. In that study, DNA from formalin-fixed, paraffin-embedded tissue was analyzed after 40 cycles of the polymerase chain reaction and compared with control specimens.

Messenger RNA coding for interleukin 5 has recently been detected in Reed-Sternberg cells and variants in patients with Hodgkin's disease. In view of a report that Epstein-Barr virus-infected cells synthesize interleukin 5, it is reasonable to speculate that the expression of the interleukin 5 gene in Reed-Sternberg cells may be related to infection by Epstein-Barr virus. The presence of interleukin 5 in Epstein-Barr virus-infected cells of Hodgkin's disease could explain the frequent presence of eosinophilia and hyperglobulinemia in patients with Hodgkin's disease. As techniques to detect the Epstein-Barr virus become more refined, it is likely that additional persons with Hodgkin's disease will be shown to be infected with this virus. Such a finding will have important implications for hematopathologists who must distinguish between benign infection by the Epstein-Barr virus and Hodgkin's disease.

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Tumor Suppressor Genes

THE HIGH FREQUENCY of cytogenetic, chromosomal, or single gene changes in most tumors had led to the concept that tumors represent a somatic cell genetic disease. With this realization, a major focus of tumor research has been to identify specific altered genetic factors and mechanisms that may be involved in tumor origin or progression.

A considerably increased understanding of tumor cell growth has come from the study of oncogenes, about 40 of which have been described. These oncogenes represent, in most instances, hyperactive deregulated states of normal cellular proto-oncogenes. Their altered states appear to be associated with increased tumor cell growth. At the cellular level, oncogenes appear to act in a dominant fashion. To date, however, mutations of the proto-oncogenes have not been associated with inherited tumors.

More recently, there has been evidence that a loss of function of certain genes may be associated with tumor growth. These have been variously called tumor suppressor genes, recessive oncogenes, antioncogenes, and growth-suppressing genes. Part of the reason for these several terms is that the normal function of these genes remains largely unknown. In contrast to the oncogenes, these tumor suppressor genes tend to be associated with tumors that are inherited in an autosomal dominant manner but that, at the tumor cell level, behave as recessive traits in that the loss of their function is associated with the tumor cell growth.

The prototype of tumor suppressor genes is the ret-